
UNIVERSITE DE LAUSANNE - FACULTE DE BIOLOGIE ET DE MEDECINE

Département de médecine
Service de néphrologie

**Effect of Sodium Loading/Depletion on Renal Oxygenation in Young
Normotensive and Hypertensive Men**

THESE

préparée sous la direction du Docteur Bruno Vogt, Privat-Doctent et
Maître d'Enseignement et de Recherche
avec la collaboration du Professeur Michel Burnier

et présentée à la Faculté de biologie et de médecine de
l'Université de Lausanne pour l'obtention du grade de

DOCTEUR EN MEDECINE

par

WG
340
PRU

Menno PRUIJM

B.MTE 3577

Médecin diplômé de la Belgique
Originaire de Rotterdam (Pays-Bas)

Lausanne

2010

Bibliothèque Universitaire
de Médecine / BIUM
CHUV-BH08 - Bugnon 46
CH-1011 Lausanne

R 0056 70260

Imprimatur

Vu le rapport présenté par le jury d'examen, composé de

Directeur de thèse Monsieur le Docteur Bruno Vogt
Co-Directeur de thèse
Expert Monsieur le Professeur Laurent Schild
Directrice de l'Ecole Madame le Professeur Stephanie Clarke
doctorale

la Commission MD de l'Ecole doctorale autorise l'impression de la thèse de

Monsieur Menno Pruijm

intitulée

*Effect of Sodium Loading/Depletion on Renal Oxygenation in
Young Normotensive and Hypertensive Men*

Lausanne, le 6 juillet 2010

*pour Le Doyen
de la Faculté de Biologie et de Médecine*



*Madame le Professeur Stephanie Clarke
Directrice de l'Ecole doctorale*

Rapport de synthèse:

Le but de cette étude était d'investiguer pour la première fois chez l'homme l'effet du sodium alimentaire et de l'hypertension artérielle sur l'oxygénation tissulaire par une technique spéciale d'imagerie à résonance magnétique nommée 'BOLD-IRM' (Blood Oxygen Level Dependent-IRM). Le BOLD-IRM est une technique nouvelle qui permet de mesurer la bio disponibilité tissulaire d'oxygène de façon non-invasive chez l'homme, en utilisant le déoxyhémoglobine comme produit de contraste endogène.

Le rational de cette étude était double. Premièrement, des changements dans l'apport sodique alimentaire devraient théoriquement influencer l'oxygénation tissulaire rénale, étant donné que la réabsorption tubulaire du sodium est un transport actif nécessitant de l'énergie et de l'oxygène. Deuxièmement, des études chez l'animal suggèrent un rôle possible de l'hypoxie tissulaire dans le développement de la néphropathie hypertensive.

Nous avons déterminé l'oxygénation rénale avec le BOLD-IRM chez dix hommes normotendus (âgés de 26.5 ± 7.4 ans) et huit hommes hypertendus non-traités (âgés de 28.8 ± 5.7 ans) une semaine après un régime riche en sel (>200 mmol/jour), et de nouveau une semaine après un régime pauvre en sel (<100 mmol/jour). En parallèle, nous avons mesuré la clearance de l'inuline, du *p*-aminohippurate (PAH) et du lithium endogène, afin de déterminer respectivement la filtration glomérulaire, le flux sanguin rénal et le 'renal sodium handling', tous des paramètres ayant la capacité d'influencer la consommation et/ou la disponibilité d'oxygène tissulaire. Nous nous attendions d'une côté à une oxygénation rénale diminuée chez les sujets hypertendus par rapport aux sujets normotendus, et d'une autre côté à une augmentation de l'oxygénation tissulaire rénale après une semaine de régime pauvre en sel par rapport à la phase d'un régime riche en sel.

Nous retenons comme résultat principal une augmentation de l'oxygénation rénale médullaire suite à une restriction sodique par rapport à un régime riche en sel chez tous les participants (normo-et hypertendus). Chez les participants normotendus ces changements correlaient avec des changements dans le transport actif du sodium, et ceci indépendamment du flux sanguin rénal. Contrairement à ce qu'on attendait, l'oxygénation rénale médullaire était plus élevée chez les sujets hypertendus par rapport aux sujets normotendus.

En résumé, ces observations offrent possiblement une explication pour les bénéfices rénaux liés à un régime pauvre en sel. En plus, la combinaison d'études de clearance et le BOLD-IRM comme utilisé dans cette étude se sont révélés un outil performant et prometteur qui peut stimuler la recherche dans ce domaine.

Remerciements:

J'aimerais tout d'abord remercier le Dr Bruno Vogt. Bruno, ton enthousiasme, ton esprit vif et brillantissime, m'ont inspiré et aidé non seulement pendant ce travail de thèse, mais aussi dans ma formation de néphrologue et de 'chercheur débutant'. Je me réjouis de me lancer dans des nouveaux projets de recherche avec toi, et j'espère que tu resteras 'BOLD' dans tous ce que tu feras.

J'aimerais également remercier de tout mon cœur le Professeur Burnier. C'est grâce à vous que j'ai pu me former en Suisse, que j'ai pu rejoindre ma femme, et que j'ai pu approfondir ma passion pour 'le sel'. Votre porte est toujours ouverte, chez vous chaque problème est le début d'une solution. Non seulement utilisez-vous l'expression d'Antoine de Saint-Exupéry, vous la vivez. 'Tout est très simple: on ne voit bien qu'avec le cœur. L'essentiel est invisible pour les yeux'.

Finalement, j'aimerais remercier Sophie, ma femme, 'soulmate' et la mère fantastique de mes deux filles Tessa et Annelys. J'ai en toi non seulement trouvé la lumière et l'amour de ma vie, mais aussi ma meilleure amie, mon source d'énergie positive et d'humour. En plus, tu m'as soutenu (et supporté) tout au long pendant ce travail de recherche, et aidé avec la rédaction. Ik hou van jou.

Hypertension

JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart
Association®



*Learn and Live*SM

Effect of Sodium Loading/Depletion on Renal Oxygenation in Young Normotensive and Hypertensive Men

Menno Pruijm, Lucie Hofmann, Marc Maillard, Sylvie Tremblay, Nicolas Glatz,
Gregoire Wuerzner, Michel Burnier and Bruno Vogt

Hypertension 2010;55;1116-1122; originally published online Mar 22, 2010;

DOI: 10.1161/HYPERTENSIONAHA.109.149682

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX
72514

Copyright © 2010 American Heart Association. All rights reserved. Print ISSN: 0194-911X. Online
ISSN: 1524-4563

The online version of this article, along with updated information and services, is
located on the World Wide Web at:

<http://hyper.ahajournals.org/cgi/content/full/55/5/1116>

Subscriptions: Information about subscribing to Hypertension is online at
<http://hyper.ahajournals.org/subscriptions/>

Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, a division of Wolters
Kluwer Health, 351 West Camden Street, Baltimore, MD 21202-2436. Phone: 410-528-4050. Fax:
410-528-8550. E-mail:
journalpermissions@lww.com

Reprints: Information about reprints can be found online at
<http://www.lww.com/reprints>

Effect of Sodium Loading/Depletion on Renal Oxygenation in Young Normotensive and Hypertensive Men

Menno Pruijm, Lucie Hofmann, Marc Maillard, Sylvie Tremblay, Nicolas Glatz, Gregoire Wuerzner, Michel Burnier, Bruno Vogt

Abstract—The goal of this study was to investigate the effect of sodium intake on renal tissue oxygenation in humans. To this purpose, we measured renal hemodynamics, renal sodium handling, and renal oxygenation in normotensive (NT) and hypertensive (HT) subjects after 1 week of a high-sodium and 1 week of a low-sodium diet. Renal oxygenation was measured using blood oxygen level–dependent magnetic resonance. Tissue oxygenation was determined by the measurement of R2* maps on 4 coronal slices covering both kidneys. The mean R2* values in the medulla and cortex were calculated, with a low R2* indicating a high tissue oxygenation. Ten male NT (mean age: 26.5±7.4 years) and 8 matched HT subjects (mean age: 28.8±5.7 years) were studied. Cortical R2* was not different under the 2 conditions of salt intake. Medullary R2* was significantly lower under low sodium than high sodium in both NT and HT subjects (28.1±0.8 versus 31.3±0.6 s⁻¹; *P*<0.05 in NT; and 27.9±1.5 versus 30.3±0.8 s⁻¹; *P*<0.05, in HT), indicating higher medullary oxygenation under low-sodium conditions. In NT subjects, medullary oxygenation was positively correlated with proximal reabsorption of sodium and negatively with absolute distal sodium reabsorption, but not with renal plasma flow. In HT subjects, medullary oxygenation correlated with the 24-hour sodium excretion but not with proximal or with the distal handling of sodium. These data demonstrate that dietary sodium intake influences renal tissue oxygenation, low sodium intake leading to an increased renal medullary oxygenation both in normotensive and young hypertensive subjects. (*Hypertension*. 2010;55:1116-1122.)

Key Words: BOLD-MRI ■ hypertension ■ sodium ■ renal tissue oxygenation ■ proximal tubule ■ medulla ■ inulin clearance

The role of sodium in the pathogenesis and treatment of essential hypertension is well established. Several population-based studies have found a strong association between sodium intake and blood pressure level, and in animals as well as in humans, low-sodium (LS) intake has proven to be an effective nonpharmacological approach to lower blood pressure.¹⁻⁴ Patients with essential and secondary forms of hypertension are often characterized by increased sodium retention either in the proximal or in the distal nephron segments.⁵⁻⁷

Experimentally, the renal handling of sodium is a process known to have an important impact on renal tissue oxygen consumption.⁸ Indeed, the tubular sodium reabsorption requires active transport systems that are energy consuming.^{9,10} Moreover, changes in sodium balance induce hormonal changes, particularly in the activity of the renin-angiotensin system that may affect renal perfusion and, hence, intrarenal tissue oxygenation distribution.^{6,11,12} Consequently, changes in sodium intake should have an effect on renal tissue oxygenation.

Until recently, quantifying changes in intrarenal oxygenation was only possible in animal models using microelectrodes and other invasive techniques.¹³ Recently, a new

technology called blood oxygen level–dependent MRI (BOLD-MRI) was developed that offers the possibility of measuring renal tissue oxygenation noninvasively in humans.^{14,15} BOLD-MRI uses deoxyhemoglobin as an endogenous contrast agent. Deoxyhemoglobin is a paramagnetic molecule that induces magnetic field perturbations in gradient echo T2*-weighted sequences. Acquisition of MRIs with increasing echo times allows for computation of their regression with the logarithm of the signal. This slope is an estimate of the relaxivity R2*, defined as 1/T2*, related to the concentration of deoxyhemoglobin. Because the concentration of blood (de)oxyhemoglobin is proportional to the Po₂ of blood, and blood Po₂ is in balance with tissue Po₂, R2* as measured by BOLD-MRI has been shown to correlate well with tissue Po₂.^{14,15}

BOLD-MRI of the kidneys has been used in different disease states, such as renal artery stenosis and renal allograft rejection, but, to our knowledge, not in essential hypertension.^{16,17} Moreover, renal tissue oxygenation has been shown to vary with age, level of hydration, diuretics, and hemoglobin levels.^{14,18} However, so far, no study has examined the role of sodium intake on renal tissue oxygenation or whether changes in external sodium load induce different trends of renal tissue oxygenation in normotensive and hypertensive

Received December 29, 2009; first decision January 15, 2010; revision accepted February 27, 2010.

From the Service of Nephrology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland.

Correspondence to Bruno Vogt, Centre Hospitalier Universitaire Vaudois, Lausanne, Service of Nephrology-Centre Hospitalier Universitaire Vaudois, Rue du Bugnon 17, 1011 Lausanne, Switzerland. E-mail bruno.vogt@chuv.ch

© 2010 American Heart Association, Inc.

Hypertension is available at <http://hyper.ahajournals.org>

DOI: 10.1161/HYPERTENSIONAHA.109.149682

individuals. On theoretical grounds, we expected to find lower $R2^*$ values, indicating higher local tissue PO_2 , under LS conditions, in both normotensive and hypertensive individuals. The goal of this study was, therefore, to investigate the impact of changes in sodium intake on renal tissue oxygenation in young male normotensive subjects and untreated age-matched hypertensive patients.

Methods

Subjects

The study was conducted in 10 male healthy, normotensive controls (NTs) and in 8 young male untreated hypertensive patients (HTs). Inclusion criteria for all of the participants were male sex, age 18 to 50 years, no history of renal disease, no illicit drug intake, and ability to understand the study protocol. Hypertensive patients could be included if they had stage I to II hypertension (blood pressure: $\geq 140/90$ and $< 180/110$ mm Hg). Baseline blood pressure of HT patients was the average daytime blood pressure as recorded during 24-hour ambulatory blood pressure measurement. For the NT group, normal blood pressure was defined as an average of 6 office measurements $\leq 135/85$ mm Hg on 2 different occasions using an automated Omron 705IT oscillometric device, as measured according to the recommendations of the European Society of Hypertension.¹⁹

Exclusion criteria for all of the participants were unstable asthma, estimated glomerular filtration rate (GFR) < 90 mL/min per 1.73 m² (using the Modification of Diet in Renal Disease formula),²⁰ anemia, and/or the presence of proteinuria on dipstick testing; a known hypersensitivity to inulin, PAH, or both; claustrophobia; and the presence of a pacemaker or other metallic implanted devices. After explaining the nature and purpose of the study, informed written consent was obtained from each subject. The protocol was approved by the local institutional review committee (Ethical Committee of the Faculty of Medicine, Lausanne).

Dietary Sodium Intake

All of the participants started with a high-sodium (HS) diet (> 150 mmol of NaCl per day) for 7 days. In the NT group, HS diet was obtained by adding 6 g of NaCl to their regular diet. The HT group followed the HS diet during the first study period by consuming salt-rich foods. The addition of 6 g of NaCl daily in this group was not permitted by the local ethical committee. Renal BOLD-MRI and renal hemodynamics were assessed in the 2 groups on day 8 using exactly the same protocol (see below). Adherence to salt intake in the LS and HS diets was verified by 24-hour urine collection on day 7. No vigorous exercise was allowed during the study period to avoid important extrarenal salt losses.

After a washout period of ≥ 1 week, the same volunteers entered the second study period and followed a LS diet for 7 days. LS diet was obtained by providing careful, identical dietary instructions (menu lists). At the end of the LS period, measurements of 24-hour urine collection, renal BOLD-MRI, and renal hemodynamics were repeated as described above.

Clearance Studies

On day 8, participants were asked to return to our research department after fasting overnight. Inulin, p-aminohippurate (PAH), and endogenous lithium clearances were performed between 8:00 and 11:30 AM, as described previously.²¹ In brief, 2 intravenous catheters were inserted into antecubital veins, one for the infusion of inulin and PAH and a second into the contralateral arm for drawing blood. Participants started with an oral water load of 5 mL/kg and continued to drink 3 mL/kg hourly throughout the clearance studies. After a 2-hour equilibration period, two 1-hour inulin and PAH clearances were obtained to measure GFR and effective renal plasma flow (ERPF), respectively. Blood pressure and heart rate were measured every 30 minutes using the same automated oscillometric device (Omron 705IT) in both HT and NT participants. Plasma renin activity, plasma aldosterone, blood urea nitrogen, creatinine, hemo-

globin, and sodium were measured at baseline and at the end of the clearance studies as described previously.²¹ Inulin was dosed by microadaptation of a diphenylamine procedure on an autoanalyzer, PAH by photometry, and lithium by electrothermal absorption spectrophotometry.²¹

Calculation of Renal Parameters

The inulin, PAH, and endogenous lithium clearances (C_x) were calculated with the formula $C_x = U_x \times V / P_x$, where U_x and P_x are urinary and plasma concentrations of the x solute, and V is the urine flow rate in milliliters per minute. Renal blood flow (RBF) was calculated as $ERPF / [1 - (\text{hematocrit}/100)]$. The fractional excretions of lithium (FE_{Li}) and sodium (FE_{Na}) were calculated using the formula $FE_x = U_x \times P_{\text{creatinine}} / (P_x \times U_{\text{creatinine}})$. Fractional distal reabsorption of sodium was estimated as $[(FE_{Li} - FE_{Na}) / FE_{Li}] / 100$. Absolute distal (postproximal) sodium reabsorption (ADR_{Na}) was estimated by the difference between the clearances of lithium and sodium multiplied by the plasma concentration of sodium.²¹

Blood Oxygen Level-Dependent MRI

BOLD-MRI was performed in the radiology department directly after the clearance studies between 1:00 and 2:00 PM on day 8 of each phase of the diet. Magnetic resonance measurements were carried out on a 3-T whole-body magnetic resonance system (Trio Tim, Siemens Medical Systems). Four coronal slices with good cortico-medullary differentiation were selected from morphological images for functional evaluation with BOLD-MRI. Twelve T_2^* -weighted images were recorded within a single breath hold of 17.4 s (in inspiration) with a modified Multi Echo Data Image Combination sequence with the following parameters: repetition time of 68 ms, echo time from 6.0 to 52.2 ms (equidistant echo time spacing: 4.2 ms), flip angle of 20°, field of view of 400×400 mm², voxel size of $1.6 \times 1.6 \times 5$ mm³, bandwidth of 700 Hz per pixel, and matrix of 256×256 . The range of echo time was limited to 52.2 ms to avoid (also for voxels with a lower signal:noise ratio) getting into the area of the Rician distribution of noise. $R2^*$ maps were calculated voxel by voxel by fitting an exponential function to the signal intensities measured for each echo time. Regions of interest were selected in the medulla and the cortex by the same experienced investigator, and a mean value of $R2^*$ index was estimated as validated and published previously. In brief, the reported mean $R2^*$ value of every participant was the mean of 4 slices, each slice with 8 regions of interest (4 in the cortex and 4 in the medulla). This technique has been shown to have a good reproducibility (mean coefficient of variance of 3% in the medulla and 4% in the cortex) for different slice directions (axial or coronal).^{22,23}

Statistical Analysis

On the basis of an expected diet-induced difference in renal oxygenation (as expressed by $R2^*$) of 10% between the 2 groups, an α of 0.05 at 2-sided significance level, and using the highest SD obtained in former studies, ≥ 8 patients per group were needed to have a power of 90%. Data are expressed as mean \pm SD. Comparisons between deoxyhemoglobin content of medullary and cortical regions for each kidney were made by using the 2-sided paired t test and differences between groups with ANOVA. Spearman rank test and linear regression were used to examine correlations. A P value < 0.05 was considered statistically significant.

Results

Normotensive Subjects

Normotensive participants (mean age: 26.5 ± 7.4 years) were whites, except one who was of Asian descent. Baseline serum creatinine was 85.9 ± 10.4 $\mu\text{mol/L}$, corresponding with an estimated creatinine clearance (estimated GFR, Modification of Diet in Renal Disease) of 103.2 ± 17.0 mL/min per 1.73 m². Changes in clinical parameters and results of clearance studies under HS and LS diets are shown in Table 1.

Table 1. Baseline Characteristics and Changes of Renal Hemodynamics in NT Individuals Under HS/LS Conditions

Parameter	NT Group, Baseline \pm SD (n=10)	HS	LS	P*
Body weight, kg	77.9 \pm 12.8	78.4 \pm 13.1	77.4 \pm 13.2	0.07
SBP, mm Hg	125 \pm 8	122 \pm 6	119 \pm 5	0.09
DBP, mm Hg	67 \pm 7	67 \pm 8	65 \pm 5	0.45
24-h urinary volume, mL		2442 \pm 903	1780 \pm 683	0.047
24-h urinary sodium, mmol		328 \pm 96	20.2 \pm 14	<0.005
P-sodium, mmol/L		139 \pm 1.4	138 \pm 1.9	0.62
Hematocrit, %		42.9 \pm 2.6	43.9 \pm 1.9	0.19
GFR, mL/min		107.9 \pm 24.9	92.7 \pm 15.7	0.03
ERPF, mL/min		725.9 \pm 187	634.4 \pm 179	0.19
RBF, mL/min		1277 \pm 344	1134 \pm 338	0.29
FF, %		15.1 \pm 2.1	15.2 \pm 3.1	0.94
FE _{Li} , %		18.7 \pm 3.9	10.3 \pm 3.7	<0.005
FE _{Na} , %		1.6 \pm 0.6	0.1 \pm 0.07	<0.005
Cl lithium, mL/min		20.3 \pm 6.6	9.4 \pm 3.0	<0.005
Cl sodium, mL/min		1.65 \pm 0.5	0.1 \pm 0.07	<0.005
ADR _{Na} , mmol/min		2.6 \pm 0.9	1.3 \pm 0.42	<0.005
PRA, ng/mL per h		0.49 \pm 0.44	1.98 \pm 0.62	<0.005
Aldosterone, pg/mL		59.5 \pm 51	296 \pm 108	<0.005

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; FF, filtration fraction; Cl, clearance; PRA, plasma renin activity. Values are shown as mean \pm SD unless otherwise specified.

*Paired *t* test was performed to compare means under HS and LS conditions.

Higher levels of GFR, ERPF, and RBF were found under HS as compared with LS conditions; filtration fraction did not change. FE_{Na}, FE_{Li}, and ADR_{Na} were, as expected, higher under the HS than the LS diet. Mean values for cortical and medullary R2*, reflecting local deoxyhemoglobin levels, are shown for each normotensive participant in Figure 1.

There were no significant changes in cortical R2* levels under HS as compared with LS conditions in the NT group (17.8 \pm 1.3 versus 18.2 \pm 0.6 s⁻¹; P=0.27). The situation was

different when analyzing the renal medulla, with R2* levels significantly higher under HS versus LS conditions (31.3 \pm 0.6 versus 28.1 \pm 0.8 s⁻¹; P<0.05) corresponding with higher local Po₂ levels under LS. Of note, the mean coefficient of variance for R2* values averaged over all of the participants between slices was 4.0% (range: 1.0% to 10.0%) in the medulla and 4.3% (range: 2.0% to 7.0%) in the cortex under HS conditions versus 4.1% (range: 1.0% to 10.0%) in the cortex and 4.7% (range: 1.0% to 9.0%) in the medulla under LS conditions.

To further investigate the role of renal sodium handling on renal tissue oxygenation, linear regression analyses were performed. Medullary R2* levels correlated positively with 24-hour urinary sodium excretion (*r*=0.55; P=0.01) and also with FE_{Na} (*r*=0.56; P=0.01), FE_{Li} (*r*=0.46; P=0.04), and ADR_{Na} (*r*=0.48; P=0.04). No correlations were found between medullary R2* levels and ERPF (*r*=0.06; P=0.8), GFR (*r*=0.29; P=0.2), or fractional distal reabsorption of sodium (*r*=0.41; P=0.08; Figure 2). There were no correlations between cortical R2* levels and renal hemodynamics or renal sodium handling.

Hypertensive Patients

Eight hypertensive subjects (mean age: 28.8 \pm 5.8 years) were included; 6 were whites and 2 were black. Baseline serum creatinine was 84.0 \pm 10.7 μ mol/L, corresponding with an estimated GFR of 106.9 \pm 15.6 mL/min per 1.73 m². All of the hypertensives were never treated, except 1 in whom treatment (β -blocker) was stopped 2 weeks before the study. Changes in clinical characteristics and clearance studies are shown in Table 2.

In HT subjects, there were no significant differences among GFR, ERPF, and RBF under LS or HS conditions. FE_{Na} was lower under LS conditions, but FE_{Li} was low, irrespective of salt intake. Changes in plasma renin activity and aldosterone levels were as expected. There was no difference between mean cortical R2* in the HT group when comparing HS with LS conditions (17.4 \pm 0.6 versus 17.8 \pm 0.9 s⁻¹; P=0.16). However, as in the NT group, medullary R2* levels were significantly lower under LS conditions (30.3 \pm 0.8 versus 27.9 \pm 1.5 s⁻¹; P<0.05; Figure

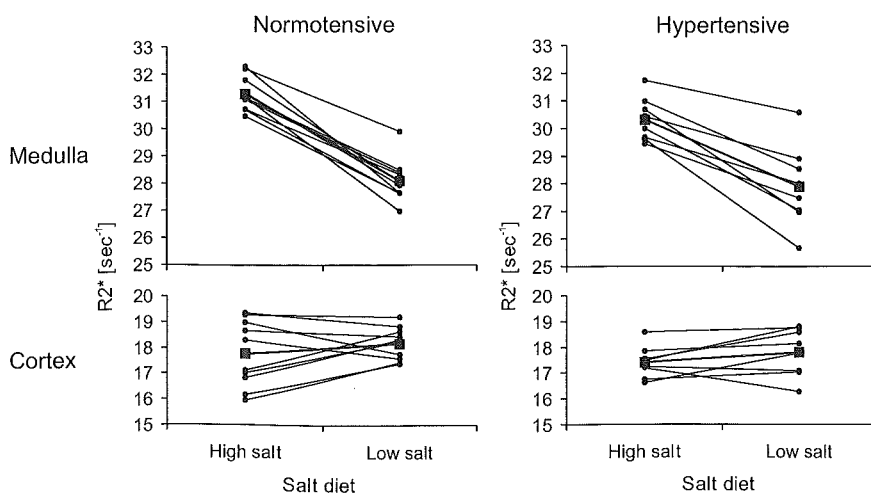


Figure 1. R2* values (in seconds⁻¹) in the medulla and cortex under HS and LS conditions for normotensive and hypertensive subjects.

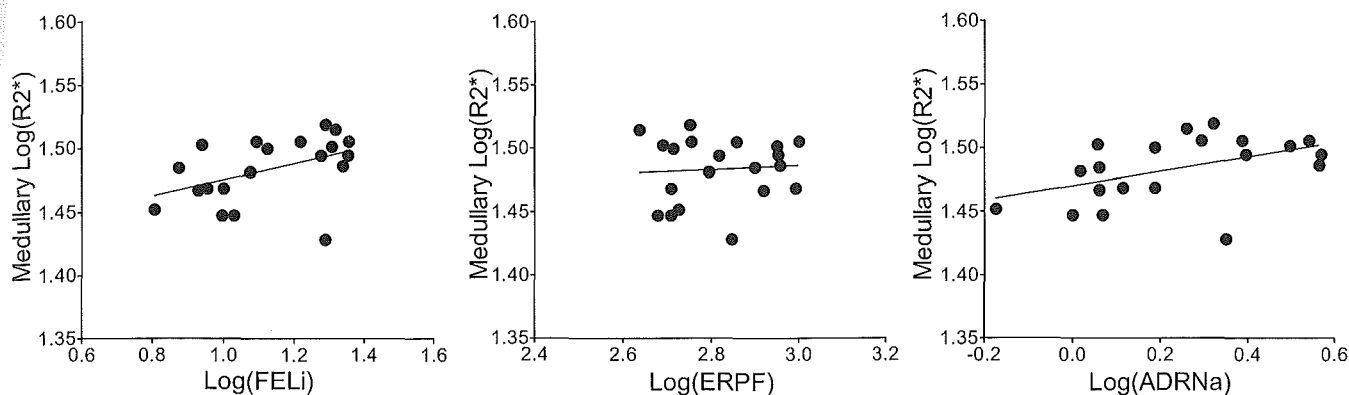


Figure 2. Scatterplots showing the relation between medullary R2* values and parameters of renal hemodynamics and renal sodium handling for normotensive subjects. Each subject is represented twice in this figure: once under HS conditions and once under LS conditions. To better approximate a normal distribution of the parameters, all of the parameters were log-transformed in this figure. The straight lines are the linear regression lines.

1). Again, dietary sodium intake, as estimated by 24-hour urinary sodium excretion, was positively correlated with medullary R2* levels ($r=0.62$; $P=0.01$). However, no correlation was found between medullary R2* levels and GFR ($r=0.03$; $P=0.91$), ERPF ($r=0.20$; $P=0.48$), FE_{Li} ($r=0.40$; $P=0.15$), or ADR_{Na} ($r=0.42$; $P=0.13$). No correlations were found between cortical R2* levels and any of the previously mentioned parameters.

Table 2. Baseline Characteristics and Changes of Renal Hemodynamics in HT Individuals Under HS/LS Conditions

Parameter	Hypertensive Group, Baseline \pm SD (n=8)	HS	LS	P*
Body weight, kg	80.8 \pm 17.6	81.8 \pm 17.4	80.9 \pm 17.8	0.045
SBP, mm Hg	142 \pm 7	141 \pm 10	136 \pm 9	0.026
DBP, mm Hg	92 \pm 5	85 \pm 12	84 \pm 12	0.39
24-h urinary volume, mL		2036 \pm 952	1718 \pm 826	0.45
24-h urinary sodium, mmol		239 \pm 126	76.8 \pm 87	<0.005
P sodium, mmol/L		139 \pm 1.3	139 \pm 0.5	0.62
Hematocrit, %		43.9 \pm 2.5	44.1 \pm 1.7	0.76
GFR, mL/min		104.6 \pm 21.8	96.4 \pm 19.3	0.35
ERPF, mL/min		572 \pm 152	567 \pm 158	0.93
RBF, mL/min		1018 \pm 272	1085 \pm 264	0.26
FF, %		19.2 \pm 4.5	17.2 \pm 3.1	0.36
FE_{Li} , %		11.4 \pm 2.0	12.4 \pm 2.4	0.43
FE_{Na} , %		1.2 \pm 0.6	0.5 \pm 0.5	<0.005
Cl lithium, mL/min		11.8 \pm 3.1	11.9 \pm 3.1	0.95
Cl sodium, mL/min		1.2 \pm 0.7	0.4 \pm 0.42	<0.005
ADR_{Na} , mmol/min		1.5 \pm 0.33	1.6 \pm 0.45	0.59
PRA, ng/mL per h		0.56 \pm 0.54	1.62 \pm 1.18	<0.005
Aldosterone, pg/mL		53.1 \pm 37.9	159 \pm 91.3	<0.005

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; FF, filtration fraction; Cl, clearance; PRA, plasma renin activity. Values are shown as mean \pm SD unless otherwise specified.

*Paired t test was performed to compare means under HS and LS conditions.

Figure 3 shows the relationship between 24-hour sodium excretion and medullary tissue oxygenation in both NT and HT subjects. Above a urinary sodium excretion of ≈ 300 mmol/d, medullary R2* appears to reach a plateau (Figure 3A). In addition, at each level of sodium excretion, medullary R2* levels were lower in HT than NT individuals (mean: 29.1 ± 1.7 versus 30.5 ± 1.7 s⁻¹; $P=0.02$), suggesting an increased tissue oxygenation in HT subjects. No difference was noted for cortical R2* levels (mean: 17.6 ± 0.8 versus 18.6 ± 1.3 s⁻¹; $P=0.11$; Figure 3B). When analyzing blood pressure as a continuous variable rather than a dichotomous separator of groups, and independently of salt intake, an inverse correlation between the mean arterial blood pressure, as measured on the days of clearance and MR studies, and medullary R2* values was found ($r=-0.37$; $P=0.026$).

Discussion

The main finding of this study is that variations in dietary sodium intake induce changes in the renal R2* signal measured by BOLD-MRI corresponding with changes in renal tissue oxygenation in male normotensive as well as hypertensive subjects. Our data demonstrate clearly that an LS intake is associated with an increased oxygenation of the renal medulla with no change in renal cortical oxygenation. In normotensive subjects, the changes in medullary oxygenation correlated with the salt-induced changes in sodium transport by the kidney but not with the changes in renal hemodynamics. In hypertension, renal medullary oxygenation correlated only with the 24-hour sodium excretion. Interestingly, at any sodium intake, medullary R2* levels were slightly lower for HT as compared with NT subjects, suggesting an increased medullary oxygenation in young subjects with mild hypertension when compared with young normotensives.

Previous studies using BOLD-MRI have shown that renal tissue oxygenation is affected by several factors, including age, level of hydration, use of diuretics, and hemoglobin levels.^{14,18} In the present study, we show for the first time in humans that dietary sodium intake also modulates renal tissue oxygenation, an LS intake increasing significantly renal medullary oxygenation without affecting the oxygenation of the renal cortex. This observation made both in normotensive

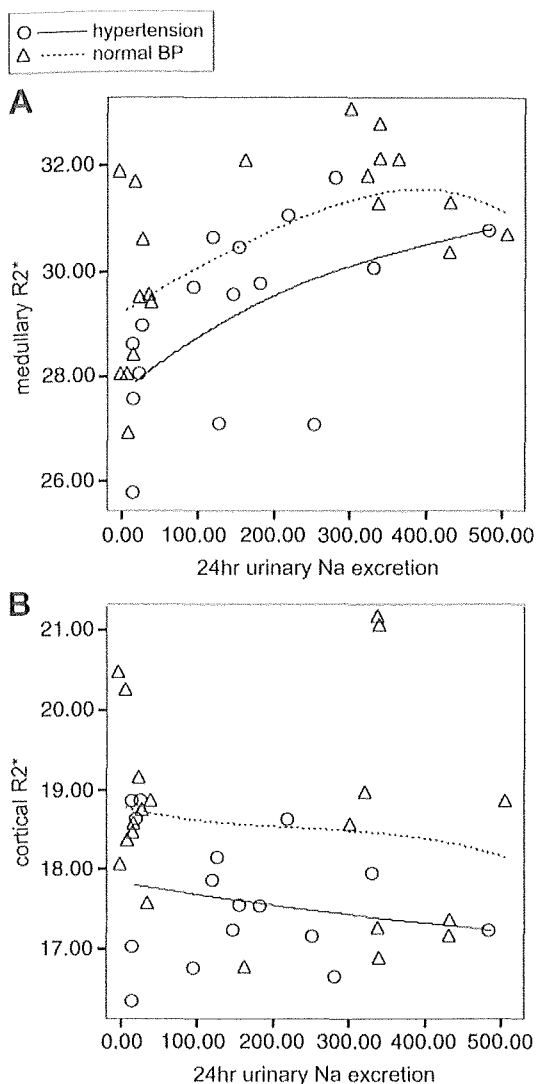


Figure 3. Comparison of (A) medullary and (B) cortical $R2^*$ signals between NT and HT subjects at different levels of salt intake, as measured by 24-hour urine excretion. Each subject is represented twice in this figure: once under HS conditions and once under LS conditions. Solid and dashed lines connect points of the HT and NT groups by local linear regression.

and young hypertensive subjects has several clinical implications. The first is that dietary sodium intake should now be considered as a potential confounder in BOLD-MRI studies. Today, water diuresis and the administration of furosemide are well recognized as factors lowering medullary $R2^*$ values.^{18,24,25} Hence, most protocols using BOLD-MRI are standardized for these factors as we did in our study. Although the effect of diuretics and water loading partly depend on dietary sodium intake,^{26,27} sodium intake has largely been ignored so far as a potential factor affecting renal tissue oxygenation. According to our data, future protocols using BOLD-MRI should either standardize for sodium intake or at least consider sodium intake as a determinant of tissue oxygenation in the analysis.

Our data also provide insights into the mechanisms involved in the regulation of renal medullary tissue oxygenation. The local tissue PO_2 is determined by the oxygen

delivery (a function of renal perfusion and blood oxygen content) and by the O_2 consumption, which is driven essentially by the GFR and the active tubular transport. More than 90% of all renal oxygen consumption is used for tubular sodium transport, which differs between the cortex and medulla. The well-perfused proximal tubules are mainly located in the renal cortex. Proximal sodium reabsorption is partly based on energy consuming active transport via basolateral Na^+,K^+ -ATPase and partly on passive transport via paracellular pathways.²⁸ Hence, despite the fact that the proximal tubules reabsorb 67% of all filtered sodium, they consume only 27% of total O_2 , resulting in local PO_2 of ≈ 50 mm Hg.^{9,10,16} In contrast, the Henle loops in the medulla receive 10% of RBF and reabsorb 30% of sodium at a relatively high energy cost. They use 67% of all O_2 , and local PO_2 is as low as 10 to 15 mm Hg under normal circumstances, which renders them susceptible to ischemic injury.^{9,10} In our study, both renal hemodynamics and renal tubular handling of sodium were measured in parallel to the determination of renal tissue oxygenation providing opportunities to correlate the salt-induced changes in renal oxygenation with the salt-induced modifications of renal perfusion and renal sodium handling. Reported medullary $R2^*$ values represent the average of higher oxygen levels in superficial medullary zones and lower PO_2 levels at deeper zones, so correlations describe trends without providing absolute regional PO_2 levels.

As expected from animal data,^{11,12} in NT subjects, medullary oxygenation was positively correlated with the 24-hour sodium excretion but also with the proximal and distal sodium reabsorption, as illustrated by the correlation between $R2^*$ values and FE_{Li} and ADR_{Na} . This suggests that, under LS conditions, medullary oxygen consumption decreases because the enhanced proximal sodium absorption (illustrated by the low FE_{Li}) leads to a reduced distal delivery of sodium and, hence, reduces the metabolic workload of medullary and distal segments of the nephron. Theoretically, lower oxygen consumption under LS conditions could have been counterbalanced by a lower blood flow because of the activation of the renin-angiotensin system leading to a lower oxygen delivery under LS conditions. In our salt-depleted subjects, a decrease in renal plasma flow and a stimulation of the renin-angiotensin system was observed but this did not reduce cortical or medullary oxygenation, and no correlation was found between medullary oxygenation and ERPF. This illustrates the potential of kidneys to preserve and even augment local PO_2 levels, independent of global RBF as a marker of oxygen delivery.

In healthy subjects, variations in dietary sodium intake did not influence renal cortical oxygenation. This is in line with other BOLD-MRI studies, showing little changes in cortical oxygenation in different situations^{17,23}; only extreme conditions, such as acute iatrogenic arterial obstruction in animal models, have been shown to significantly alter cortical $R2^*$.²⁹ This is explained by the technical limitation of BOLD-MRI for renal cortex analyses. BOLD-MRI is less sensitive to changes in cortical PO_2 , because cortical blood PO_2 lies on the shoulder of the hemoglobin oxygenation curve, in contrast to medullary PO_2 , which lies on the linear part of the curve, and

is more sensitive to small changes in oxygen tension. Thus, larger differences in local PO_2 are necessary to observe similar changes in the $R2^*$ signal for the cortex as compared with the medulla.¹⁴

In young hypertensive patients, a significant increase in medullary oxygenation was found in response to the LS diet. The changes in medullary oxygenation correlated with the 24-hour urinary sodium excretion, but in contrast to normotensive subjects, no correlation was found with segmental renal sodium handling. Moreover, no correlation was found between medullary $R2^*$ values and all of the tested parameters of global renal hemodynamics. Although we do not have a clear explanation for the lack of association of sodium transport and oxygen consumption in these young hypertensives, some potential explanations might be proposed. The first is the rather small number of hypertensive subjects included in the study. The second is the lack of changes in FE_{Na} when the diet was changed from HS to LS. We have reported previously that FE_{Na} remains inappropriately low in some hypertensive patients, reflecting an enhanced proximal sodium reabsorption and salt sensitivity.⁷ A third possible factor is the smaller decrease in sodium excretion under the LS diet in the hypertensive participants. Although the same dietary regimen was applied to normotensive and hypertensive subjects, the compliance of the latter appears to be less than that of the former. At last, the rise in medullary PO_2 in HT subjects could be explained by changes in renal microcirculation with an intrarenal redistribution of blood flow. However, this hypothesis remains to be confirmed, with more precise measurements of intrarenal blood flow.

On the basis of animal studies, we merely expected to find higher $R2^*$ levels in the HT as compared with the NT group, reflecting more intense medullary hypoxia, both under HS and LS diets.³⁰ Although our subjects and patients were well matched for several parameters, we rather found an increased renal tissue oxygenation in our young hypertensive patients, as presented in Figure 3 and as illustrated by the inverse relationship between blood pressure as a continuous trait and medullary $R2^*$ values.

Studies in hypertensive rats using oxygen microelectrodes found pronounced medullary hypoxia as compared with normotensive controls^{10,31}; this has been linked to reactive oxygen species, disturbed renal microcirculation, and inefficient use of oxygen for tubular transport. To the best of our knowledge, no other studies have examined renal oxygenation in humans with essential hypertension using BOLD-MRI. One BOLD-MRI study has been performed in hypertensive patients experiencing renal artery stenosis.²⁴ In this context, normal-sized kidneys with high-grade artery stenosis had high medullary $R2^*$ levels at baseline that fell after the administration of furosemide. Atrophic kidneys with high-grade artery stenosis had reduced $R2^*$ values at baseline that did not change after furosemide. A possible explanation for the lower medullary $R2^*$ in the HT group in our study could, thus, be increased tissue oxygen content because of less active medullary sodium transport, as an adaptation to earlier medullary hypoxia, for example, in the form of hypoxia inducible factor-induced interstitial fibrosis, or adaptations in medullary microcirculation. Recent data from rabbit studies

suggest that renal tissue PO_2 is relatively independent of RBF, possibly because preglomerular diffusional shunting of oxygen from arteries to veins decreases with decreasing RBF and vice versa.³² It is, thus, possible that HT subjects present altered arterial-venous shunting as compared with NT subjects. However, this remains hypothetical, because our data on renal hemodynamics provided information on the total renal perfusion and filtration rate but not on renal microcirculation. At last, it is important to note that the study was conducted in young men with mild untreated hypertension. It is conceivable that different patterns of renal oxygenation might be seen in long-standing or more severe forms of hypertension.

Perspectives

This study demonstrates that an LS intake leads to increased renal medullary oxygenation as compared with HS intake in normotensive as well as in hypertensive subjects. This finding might have some clinical implications. First, it demonstrates the importance of considering sodium intake as a potential confounding factor in BOLD-MRI analyses, and it illustrates the high sensitivity of this technique to detect changes in renal oxygen content. It also provides additional positive arguments for the recommendation of an LS intake in hypertension, although the potential role of an LS intake on renal tissue oxygenation and, hence, on the progression of chronic renal diseases in normotensive and hypertensive patients, remains to be documented. Nonetheless, it has been shown that renal hypoxia is associated with the progression of several renal disease states, such as diabetes mellitus, renal artery stenosis, and analgesic nephropathy.¹³ However, this does not necessarily imply that high local medullary PO_2 values are renoprotective. To the best of our knowledge, no studies have examined the prognostic value of local PO_2 levels in humans. Prospective studies including other age groups with different comorbidities are, thus, needed to assess whether the beneficial effects of an LS diet can be explained by its impact on renal tissue oxygenation.

Acknowledgments

We thank Reto Meuli for his expert advice and technical supervision of BOLD-MRI exams.

Sources of Funding

The study was supported by a grant of the Swiss Renal Foundation, by the Hypertension Research Foundation, by the Swiss National Science Foundation (FN 33CM30-124087 and FN 3100AO-120406) and by the Centre D'imagerie Biomédicale" of the University of Lausanne, the Swiss Federal Institute of Technology Lausanne, the University of Geneva, the Centre Hospitalier Universitaire Vaudois, the Hôpitaux Universitaires de Genève, and the Leenaards and the Jeantet foundations.

Disclosures

None.

References

1. Khaw KT, Bingham S, Welch A, Luben R, O'Brien E, Wareham N, Day N. Blood pressure and urinary sodium in men and women: the Norfolk Cohort of the European Prospective Investigation Into Cancer (EPIC-Norfolk). *Am J Clin Nutr*. 2004;80:1397-1403.

2. Intersalt Cooperative Research Group. Intersalt: an international study of electrolyte excretion and blood pressure: results for 24 hour urinary sodium and potassium excretion. *BMJ*. 1988;297:319-328.
3. He FJ, MacGregor GA. Effect of longer-term modest salt reduction on blood pressure. *Cochrane Database Syst Rev*. 2004;3:CD004937.
4. Elliott P, Walker LL, Little MP, Blair-West JR, Shade RE, Lee DR, Rouquet P, Leroy E, Jeunemaitre X, Ardaillou R, Paillard F, Meneton P, Denton DA. Change in salt intake affects blood pressure of chimpanzees: implications for human populations. *Circulation*. 2007;116:1563-1568.
5. Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. *Cell*. 2001;104:545-556.
6. Chioloro A, Maillard M, Nussberger J, Brunner HR, Burnier M. Proximal sodium reabsorption: an independent determinant of blood pressure response to salt. *Hypertension*. 2000;36:631-637.
7. Burnier M, Bochud M, Maillard M. Proximal tubular function and salt sensitivity. *Curr Hypertens Rep*. 2006;8:8-15.
8. Epstein FH. Oxygen and renal metabolism. *Kidney Int*. 1997;51:381-385.
9. Brezis M, Rosen S, Silva P, Epstein FH. Renal ischemia: a new perspective. *Kidney Int*. 1984;104:375-383.
10. Welch WJ. Intrarenal oxygen and hypertension. *Clin Exp Pharmacol Physiol*. 2006;33:1002-1005.
11. Stillman IE, Brezis M, Heyman SN, Epstein FH, Spokes K, Rosen S. Effects of salt depletion on the kidney: changes in medullary oxygenation and thick ascending limb size. *J Am Soc Nephrol*. 1994;4:1538-1545.
12. Mercer PF, Zusko EA. Whole kidney and cortical blood flow in rats on high and low sodium diets. *J Physiol*. 1974;237:495-504.
13. Heyman SN, Khamaisi M, Rosen S, Rosenberger C. Renal parenchymal hypoxia, hypoxia response and the progression of chronic kidney disease. *Am J Nephrol*. 2008;28:998-1006.
14. Prasad PV. Evaluation of intra-renal oxygenation by BOLD MRI. *Nephron Clin Pract*. 2006;103:c58-c65.
15. Prasad PV, Edelman RR, Epstein FH. Noninvasive evaluation of intrarenal oxygenation with BOLD MRI. *Circulation*. 1996;94:3271-3275.
16. Gomez SI, Warner L, Haas JA, Bolterman RJ, Textor SC, Lerman LO, Romero JC. Increased hypoxia and reduced renal tubular response to furosemide detected by BOLD magnetic resonance imaging in swine renovascular hypertension. *Am J Physiol Renal Physiol*. 2009;297:F981-F986.
17. Sadowski EA, Djamali A, Wentland AL, Muehrer R, Becker BN, Grist TM, Fain SB. Blood oxygen level-dependent and perfusion magnetic resonance imaging: detecting differences in oxygen bioavailability and blood flow in transplanted kidneys. *Magn Reson Imaging*. 2010;28:56-64.
18. Prasad PV, Epstein FH. Changes in renal medullary pO₂ during water diuresis as evaluated by blood oxygenation level-dependent magnetic resonance imaging: effects of aging and cyclooxygenase inhibition. *Kidney Int*. 1999;55:294-298.
19. O'Brien E, Asmar R, Beilin L, Imai Y, Mallion JM, Mancia G, Mengden T, Myers M, Padfield P, Palatini P, Parati G, Pickering T, Redon J, Staessen J, Stergiou G, Verdecchia P. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. *J Hypertens*. 2003;21:821-848.
20. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation—Modification of Diet in Renal Disease Study Group. *Ann Intern Med*. 1999;130:461-470.
21. Burnier M, Rutschmann B, Nussberger J, Versaggi J, Shahinfar S, Waeber B, Brunner HR. Salt-dependent renal effects of an angiotensin II antagonist in healthy subjects. *Hypertension*. 1993;22:339-347.
22. Simon-Zoula SC, Hofmann L, Giger A, Vogt B, Vock P, Frey FJ, Boesch C. Non-invasive monitoring of renal oxygenation using BOLD-MRI: a reproducibility study. *NMR Biomed*. 2006;19:84-89.
23. Hofmann L, Simon-Zoula S, Nowak A, Giger A, Vock P, Boesch C, Frey FJ, Vogt B. BOLD-MRI for the assessment of renal oxygenation in humans: acute effect of nephrotoxic xenobiotics. *Kidney Int*. 2006;70:144-150.
24. Textor SC, Glockner JF, Lerman LO, Misra S, McKusick MA, Riederer SJ, Grande JP, Gomez SI, Romero JC. The use of magnetic resonance to evaluate tissue oxygenation in renal artery stenosis. *J Am Soc Nephrol*. 2008;19:780-788.
25. Tumkur SM, Vu AT, Li LP, Pierchala L, Prasad PV. Evaluation of intra-renal oxygenation during water diuresis: a time-resolved study using BOLD MRI. *Kidney Int*. 2006;70:139-143.
26. Burnier M, Monod ML, Chioloro A, Maillard M, Nussberger J, Brunner HR. Renal sodium handling in acute and chronic salt loading/depletion protocols: the confounding influence of acute water loading. *J Hypertens*. 2000;18:1657-1664.
27. Beutler JJ, Boer WH, Koomans HA, Dorhout Mees EJ. Renal hemodynamic and tubular response to furosemide in man during normal and restricted sodium intake. *Nephron*. 1990;54:208-213.
28. Kiil F. Renal energy metabolism and regulation of sodium reabsorption. *Kidney Int*. 1977;11:153-160.
29. Alford SK, Sadowski EA, Unal O, Polzin JA, Consigny DW, Korosec FR, Grist TM. Detection of acute renal ischemia in swine using blood oxygen level-dependent magnetic resonance imaging. *J Magn Reson Imaging*. 2005;22:347-353.
30. Li LP, Li BS, Storey P, Fogelson L, Li W, Prasad P. Effect of free radical scavenger (tempol) on intrarenal oxygenation in hypertensive rats as evaluated by BOLD MRI. *J Magn Reson Imaging*. 2005;21:245-248.
31. Rosenberger C, Rosen S, Heyman SN. Renal parenchymal oxygenation and hypoxia adaptation in acute kidney injury. *Clin Exp Pharmacol Physiol*. 2006;33:980-988.
32. Leong CL, Anderson WP, O'Connor PM, Evans RG. Evidence that renal arterial-venous oxygen shunting contributes to dynamic regulation of renal oxygenation. *Am J Physiol Renal Physiol*. 2007;292:F1726-F1733.